

# Compatibility relations between the edible carrot *Daucus carota* and *D. pusillus*, a related wild species from the Argentinian Pampas

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**Abstract** To establish the feasibility of hybridization between the wild carrot species *Daucus pusillus* Michx. ( $2n = 2x = 22$ ;  $2n = 2x = 22$  and  $20$ ), collected in the pampas grasslands of Argentina, and the edible carrot, *Daucus carota* L. ( $2n = 2x = 18$ ), controlled pollinations were attempted on the plant. Due to the difficulties encountered, flowers of 12 accessions and three commercial cultivars were excised from individual plants and pollinated in Petri dishes following an incomplete diallel design. After processing, the pollinated pistils (four to six per genotypic combination) were observed under a microscope with UV light. Pollen tubes reaching the ovaries and/or the ovules (compatible relation) were observed in six out of nine *D. pusillus* × *D. pusillus* and seven out of 18 *D. pusillus* × *D. carota* genotypic combinations. In

the eight *D. carota* × *D. pusillus* genotypic combinations, only ungerminated pollen, pollen not adhered to the stigmas or pollen tubes overlapping the stylar tissue were observed. Additional flowers were pollinated in a sample of compatible genotypic combinations and the pollinated pistils were in vitro culture to study embryo and endosperm development. Eight out of nine pistils from the intraspecific and nine out of 13 from the interspecific crosses enlarged to form apparently normal schizocarps. Histological analyses revealed normal development of embryo and endosperm. The breeding barriers between the two species are incomplete, making feasible the obtainment of interspecific hybrids by conventional techniques.

**Keywords** *Daucus carota* L. · *D. pusillus* Michx. · pre-zygotic and post-zygotic breeding barriers

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## Introduction

Carrot (*Daucus carota* L. var. *sativus* Hoffm.,  $2n = 2x = 18$ ) is the major vegetable umbellifer cultivated worldwide. It has an estimated number of around 25 related wild species (Rubatzky et al. 1999). Its center of origin is Central Asia, but the major genetic variability for the genus can be found in the Mediterranean region, particularly in North Africa.

For the Argentinian flora, *Daucus montevidensis* Link ex Sprengel has been cited as a common species in sandy soils of Buenos Aires province (Cabrera

1953), growing in the “flechillar”, a plant community of indigenous grasses (*Stipa*, *Aristida*, *Piptochaetium*) in high and fertile fields of the Salado basin (Vervoorst 1967), and in the provinces of Córdoba, Entre Ríos, and Corrientes (Marzocca 1957). Notwithstanding, and for the same type of soil, Cabrera (1965) and Cabrera and Zardini (1978) have described *Daucus pusillus* Michx. as a synonym of *D. montevidensis*. On the other hand, *D. pusillus* has been cited as a temperate climate species of both South and North America, with  $x = 11$ ,  $10$  (?) (Correa Maevia 1988). In Argentina, *D. pusillus* usually grows in dry and sandy soils in Patagonia (Correa Maevia 1988). According to Lincoln Constance (Correa Maevia 1988), *D. pusillus* is a synonym of both *D. montevidensis* and *D. hispidifolius*. Clos.

More recently, Camadro et al. (2007) sampled 30 *Daucus* populations in the pampas grasslands of two Argentinian provinces, Buenos Aires and Entre Ríos. These populations were distinguishable by their morphological characters, chromosome numbers and adaptation to paradigmatic habitats. Thus, they were unmistakably assigned to: (1) wild *D. carota* ( $2n = 2x = 18$ ) and (2) *D. pusillus* ( $2n = 2x = 22$ ;  $2n = 2x = 22$  and  $20^1$ ). The latter designation would be *D. pusillus* or *D. montevidensis* if two different taxa were recognized. Based on the large morphological variability observed in the natural sampled populations, herbarium specimens and published drawings, they decided to follow the nomenclature of Cabrera (1965), Cabrera and Zardini (1978) and Lincoln Constance (in Correa Maevia 1988), and tentatively considered all the accessions with  $2n = 2x = 22$ ;  $2n = 2x = 22$  and  $20$  as *D. pusillus* until molecular studies are carried out.

*Daucus carota* is an allogamous species, susceptible to pest and diseases. Technological and commercial motives have increased the demand of cultivar uniformity and, thus, the need of developing hybrid cultivars (Rubatzky et al. 1999). Two distinct sources of genic-cytoplasmic male sterility (CMS) have provided a system for commercial hybrid seed production: petaloid and brown anther. The USA relies almost entirely on the first type, in contrast to Europe, due to the differences in the stability of expression of the genes controlling the trait upon

environments (Rubatzky et al. 1999). The dependence of a single source of CMS can lead to genetic vulnerability. A clear example was the devastating *Helminthosporium maydis* race T epiphytia that, in the USA, affected seed production of hybrid maize with T-type CMS (Scheifele et al. 1970). Wild *Daucus* germplasm could be a valuable potential source of genes controlling resistance/tolerance to adverse biotic and abiotic factors as well as male sterility and desirable culinary, nutritional and processing traits, among others. To put this germplasm into a useful form for breeding, it is necessary to determine first, if the species are sexually compatible. The objective of this work was, then, to investigate pollen–pistil relations in intra- and interspecific controlled crosses between *D. pusillus* and *D. carota* and the eventual seed formation if compatible combinations were detected.

## Materials and methods

Inflorescences of three commercial cultivars of *D. carota* grown in the experimental field in Balcarce, Conquista, Criolla INTA and Larga Cordobesa, and of individual plants of *D. pusillus*, either collected in their natural habitats at bloom or grown in a greenhouse from seeds previously collected in those habitats (Table 1), were brought into the laboratory and placed into water jars. In addition, individual plants of *D. pusillus* were left in isolation to determine their type of reproduction (allogamy vs autogamy).

Controlled intraspecific *D. pusillus* and reciprocal interspecific *D. pusillus*–*D. carota* crosses were attempted on individual plants. To evaluate pollination and subsequent events more closely, individual flowers close to anther exertion were placed under a stereo-microscope. With the aid of tweezers and a histological needle, anthers were removed and fresh pollen was placed on the stigmata. Pollinated pistils were then placed in Petri dishes with humid filter paper, at room temperature. Forty-eight hours after pollination, they were transferred to excavated glass slides and processed according to Martin (1958) with the aid of a micropipette to add and remove the chemical solutions. Stained pistils were then placed in a drop of glycerine on a glass slide, squashed with a cover slip and observed under a microscope with

<sup>1</sup> Some plants with around 90% of the cells with  $2n = 22$  and 10% of the cells with  $2n = 20$  (aneusomaty)

**Table 1** Accessions of *Daucus pusillus* and their collection sites in Buenos Aires and Entre Ríos provinces, Argentina

Accession	Province and area Buenos Aires	Locality	Coordinates
ECMC pus 5	<i>Salado Basin</i>	25 de Mayo	35° 19' 13.50''S 60° 06' 7.86''W
ECMC pus 1	<i>Sierra de la Ventana</i>	In front of Hotel Provincial	38° 13' 55.57''S 61° 52' 10.64''W
ECMC pus 2	<i>Sierra de la Ventana</i>	Abra El Pantanoso	38° 07' 20.47''S 61° 45' 33.68''W
ECMC pus 3	<i>Sierra de la Ventana</i>	Cerro Bahía Blanca	38° 09' 35.13''S 61° 54' 44.81''W
EMC pus 13	<i>Gral Madariaga</i>	Mar Azul	37° 19' 60.00''S 57° 00' 00.00''W
EMC pus 14	<i>Gral Madariaga</i>	Mar de las Pampas	37° 19' 23.23''S 57° 01' 05.42''W
EMC pus 15	<i>Gral Madariaga</i>	Villa Gesell	37° 14' 07.00''S 57° 01' 45.20''W
ECMC pus 7	<i>Samborombón Bay</i>	Route 11, km 22	36° 17' 13.48''S 57° 10' 42.48''W
ECMC pus 8	<i>Samborombón Bay</i>	Route 11, km 162	36° 25' 20.50''S 58° 06' 04.96''W
ECMC pus 1	<i>Samborombón Bay</i>	Route 11, Stream El Porteño	36° 17' 57.46''S 57° 07' 56.68''W
ECMC pus 11	<i>Confluence rivers Paraná and Río de la Plata</i>	Martín García Island	34° 10' 55.92''S 58° 15' 02.88''W
ECMC pus 12	<i>Entre Ríos South</i>	Médanos	33° 25' 37.91''S 59° 04' 46.66''W

UV light. If compatible genotypic combinations were identified, additional pistils were pollinated and cultured on MS medium (Murashige and Skoog 1962) for eventual seed recovery.

To study embryo and endosperm development, the cultured pistils were removed from the culture medium five weeks after culture initiation, fixed in FAA (1 formaline: 8 ethanol: 1 acetic acid, v/v/v), processed according to Sass (1952) and cut with a rotary microtome to obtain 10 µm width serial sections.

## Results

The plants of *D. pusillus* grown in isolation—in the absence of apparent pollen vectors—set abundant seeds, indicating that their reproduction is autogamous. In contrast, *D. carota* is known to be an insect-

pollinated allogamous species, and its intraspecific pollination capacity was not evaluated in this study.

Microscopic evaluations of controlled pollinations are presented in Table 2. Four to six pistils were analyzed for each of the listed intra- and interspecific genotypic combination (nine of intra-*D. pusillus*, 18 of *D. pusillus* × *D. carota* and eight of *D. carota* × *D. pusillus*). In the intraspecific crosses, pollen tubes reaching the ovaries and/or the ovules (compatible relation) were observed in six of the nine genotypic combinations, that included reciprocals (Fig. 1a). In the remaining three combinations, only ungerminated pollen, few pollen tubes and pollen tubes that did not reach the ovary, respectively, were observed. In the *D. pusillus* × *D. carota* crosses, seven genotypic combinations were compatible in at least one pistil (Fig. 1b). In three other combinations, a few pollen tubes were observed in at least one style. In addition, abundant ungerminated pollen grains

**Table 2** Pollen–pistil relations in intra-specific *Daucus pusillus* and interspecific *D. pusillus*–*D. carota* crosses and enlargement of in vitro cultivated pistils of compatible genotypic combinations

Cross	Pollination observations	Number of pistils enlarged after pollination and in vitro culture
<i>D. pusillus</i> × <i>D. Carota</i>		
ECMC pus 2 (7) <sup>a</sup> × Conquista	Compatibility <sup>b</sup>	
ECMC pus 7 (5) × Larga Cordobesa	Many ungerminated pollen grains; one pollen tube in style	
ECMC pus 7 (5) × Conquista	Many ungerminated pollen grains	
ECMC pus 11 (7) × Conquista	Compatibility	4 (3) <sup>c</sup>
ECMC pus 11 (7) × Larga Cordobesa	(Not evaluated)	3 (3)
ECMC pus 3 (1) × Conquista	In one pistil out of four, compatibility	
ECMC pus 15 (2) × Conquista	One very long pollen tube; in three, only ungerminated pollen	
ECMC pus 13 (1) × Conquista	Compatibility	
ECMC pus 13 (1) × Criolla	Compatibility	
ECMC pus 12 (3) × Conquista	Many ungerminated pollen grains (only one germinated)	5 (3, moderately)
ECMC pus 12 (3) × Larga Cordobesa	Many ungerminated pollen grains; compatibility in one pistil	
ECMC pus 12 (3) × Criolla INTA	Some long pollen tubes (as in the reciprocal cross)	
ECMC pus 5 (1) × Larga Cordobesa	One pistil apparently compatible; three with ungerminated pollen	1 albino
ECMC pus 15 (8) × Larga Cordobesa	Ungerminated pollen, mass of pollen grains and tubes; disrupted structures	
ECMC pus 15 (8) × Criolla INTA	Many ungerminated pollen grains, some sterile, and broken stigmata	
ECMC pus 10 (7) × Conquista	Ungerminated pollen	
ECMC pus 10 (7) × Larga Cordobesa	Ungerminated pollen	
ECMC pus 10 (7) × Criolla INTA	Ungerminated pollen	
<i>D. carota</i> × <i>D. pusillus</i>		
Larga Cordobesa × ECMC pus 12 (3)	Many ungerminated pollen grains, not on pistils	
Larga Cordobesa × ECMC pus 7 (5)	Many ungerminated pollen grains, not on pistils	
Conquista × ECMC pus 7 (5)	Many ungerminated pollen grains	
Conquista × ECMC pus 12 (3)	Many ungerminated pollen grains, not on pistils	
Conquista × ECMC pus 10 (7)	Many ungerminated pollen grains not adhered to pistils	
Conquista × ECMC pus 15 (8)	Few pollen grains, ungerminated	
Criolla × ECMC pus 14 (7)	Many pollen grains, ungerminated	
Criolla × ECMC pus 12 (3)	Many long pollen tubes, overlapping the stylar tissue, not seen entering the ovary	
<i>D. pusillus</i> × <i>D. pusillus</i>		
ECMC pus 12 (3) × ECMC pus 2 (7)	Two pollen tubes in two pistils	6 (6)
ECMC pus 2 (7) × ECMC pus 12 (3)	Compatibility	
ECMC pus 7 (5) × ECMC pus 2 (5)	Compatibility, highly variable among pistils	
ECMC pus 2 (5) × ECMC pus 10 (7)	Compatibility	
ECMC pus 7 (5) × ECMC pus 12 (3)	Pollen tubes in styles, not seen in ovaries	
ECMC pus 12 (3) × ECMC pus 7 (5)	Ungerminated pollen grains	

**Table 2** continued

Cross	Pollination observations	Number of pistils enlarged after pollination and in vitro culture
ECMC pus 11(7) × ECMC pus 10 (7)	Compatibility	
ECMC pus 11(7) × ECMC pus 8 (1)	One pollen tube in style; one pollen tube close to the ovule	
ECMC pus 11 (7) × ECMC pus 1 (1)	Compatibility	3 (2)

<sup>a</sup> Parenthesized numbers designate individual *D. pusillus* plants for an accession

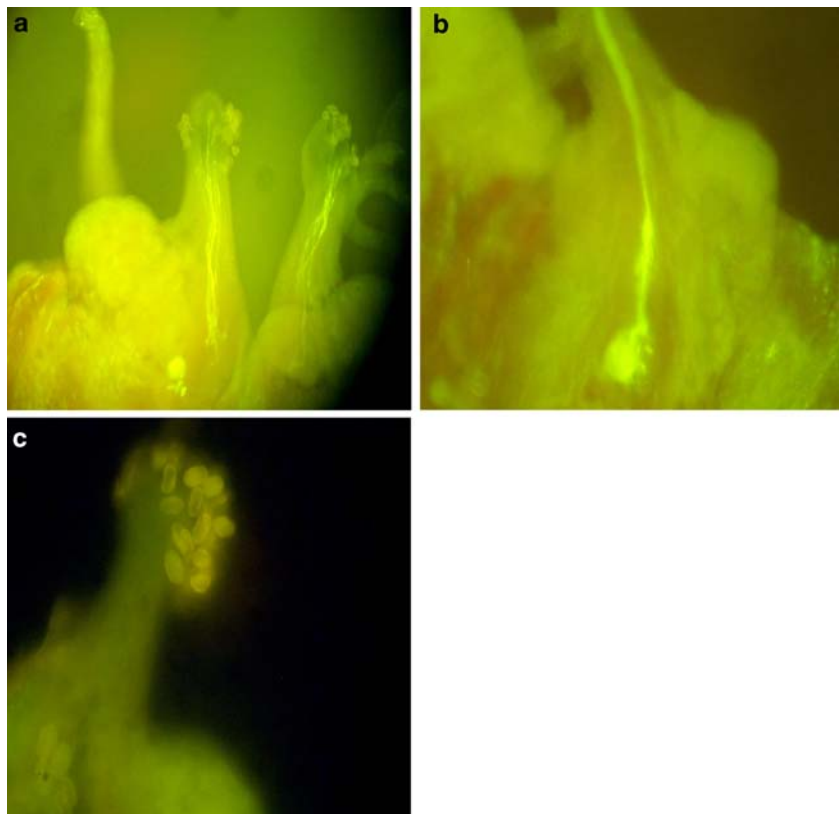
<sup>b</sup> Pollen tubes reaching the ovaries and/or the ovules

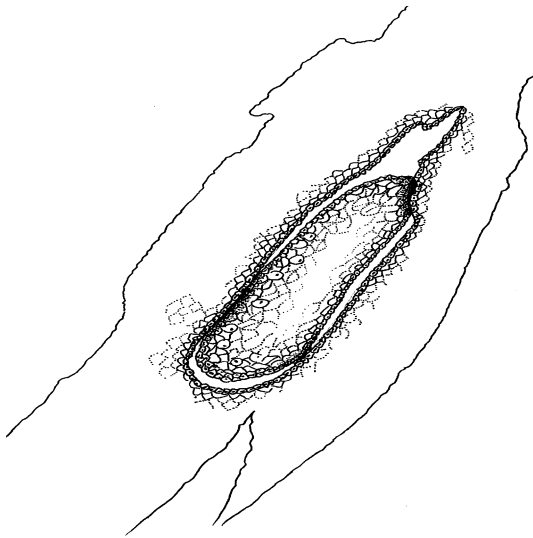
<sup>c</sup> Parenthesized values indicate the number of enlarged pistils one month after culture initiation

were observed in seven of the remaining combinations and one was not evaluated. In contrast, only abundant ungerminated pollen grains on or around the stigmata (not adhered) and pollen tubes overlapping but not penetrating the stylar tissue were observed in the eight *D. carota* × *D. pusillus* combinations (Fig. 1c). Eight out of nine in vitro cultured pollinated pistils derived from intraspecific *D. pusillus* and nine out of 13 of those derived from

interspecific *D. pusillus* × *D. carota* crosses (including three from a genotypic combination that had not been analyzed at the pollen–pistil level) enlarged to form what appeared to be normal schizocarps. The histological observations revealed normal embryo and endosperm growth in the first type of cross and embryos at the globular or more advanced stages accompanied by normal endosperm, in the second one (Fig. 2).

**Fig. 1** Pollen–pistil compatible relations. (a) intraspecific *Daucus pusillus* cross, ECMC pus 11 (7) × ECMC pus 1 (1); (b) interspecific *D. pusillus* × *D. carota* cross, ECMC pus 11 (7) × Conquista; (c) interspecific *D. carota* × *D. pusillus* cross, Conquista × ECMC pus 7 (5)





**Fig. 2** Normal endosperm development in interspecific *D. pusillus*  $\times$  *D. carota* cross, ECMC pus 11 (7)  $\times$  Conquista

## Discussion

*Daucus pusillus* is a species that has not been either genetically or agronomically characterized. In Argentina, it has been reported for a wide range of macroenvironments, from dry and cold in Southern Buenos Aires province to humid and warm in Southern Entre Ríos province (Camadro et al. 2007), always growing in sandy soils. Due to its wide adaptation, it could be a potential valuable source of genes of interest for breeding, including resistance/tolerance to adverse biotic and abiotic factors as well as male sterility for hybrid seed production.

The flowering period of *D. carota* and *D. pusillus* partially overlapped, but was much shorter in *D. pusillus*. This fact hindered the interspecific crossing work because most plants of the wild species produced only one or two inflorescences and all flowers matured within a week. In addition, it was very difficult to emasculate individual flowers of *D. pusillus*—even under a glass magnifier—without inflicting damage to the pistils because these flowers are very small (approximately 1–1.5 mm long). Thus, they had to be detached from the umbels and emasculated under a stereo-microscope. This manipulation plus the processing of the pollinated pistils on excavated glass slides proved to be very useful to study the pollen–pistil compatibility

relations. However, for the final objective of investigating seed formation, we have established that in vitro manipulation of the pollinated pistils will be required.

Abundant ungerminated pollen grains on or near the stigma or in their surroundings were observed in only one of the intraspecific genotypic combinations but was frequent in the interspecific ones. This could be due either to the lack of receptiveness of the stigma at the time of pollination or to inappropriate pollen placement on the stigma, rather than to an incompatible reaction. Thus, the pollination technique has to be adjusted to rule out the first two possibilities. Moreover, the number of interspecific genotypic combinations that could be made in the *D. carota*  $\times$  *D. pusillus* direction was only 53% of those made in the reciprocal direction. Therefore, no conclusions can be drawn regarding the compatibility relations in that direction of the cross.

In vitro culture of pollinated pistils was evaluated only after a compatible combination was identified, at which time there were rarely remaining flowers in the appropriate stage to repeat the cross. Consequently, only a low number of pollinated pistils were in vitro cultured. Notwithstanding, embryo and endosperm development in the intra- and interspecific (one direction) genotypic combinations was observed. As *D. carota* and *D. pusillus* appear to be separated mainly by incomplete pre-zygotic barriers, it seems feasible to obtain hybrid seed in controlled crosses between compatible genotypes. If the seed were viable, then hybrid plants could be obtained for breeding purposes. Towards this end, either a technique for successful pollinations on the plant or the development of medium composition for in vitro culture of detached pollinated flowers will be needed.

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